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Latex Agglutination Test for Staphylococcal Toxic Shock Syndrome Toxin 1

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A reversed passive latex agglutination method, in which latex particles were sensitized with specific anti-toxic shock syndrome toxin-1 (TSST-1) immunoglobulin, was found to be a simple and sensitive method for the detection of TSST-1 production by *Staphylococcus aureus* strains. The minimum amount of TSST-1 detectable was approximately 1.0 ng/ml. Of 41 *S. aureus* isolates from toxic shock syndrome patients and controls, 23 were positive for TSST-1 production, whereas only 20 strains were positive for TSST-1 production by an Ouchterlony immunodiffusion method. The reversed passive latex agglutination method was used to examine *S. aureus* strains isolated in Japan from staphylococcal infections, feces from healthy individuals, food from poisoning outbreaks, and market food.

Toxic shock syndrome (TSS), a clinical entity first described by Todd et al. (15), is a disease resulting from various types of staphylococcal infection (10), the majority of which have been associated with the use of tampons during menstruation (6, 14). Over 90% of the staphylococcal strains isolated from TSS cases were found to produce a common toxin (2, 4, 13) which has been given the name TSS toxin-1 (TSST-1) (3). The methods most commonly used for the detection of TSST-1 are some type of gel diffusion test with specific antisera. One of the simplest of these methods is the optimum-sensitivity plate (OSP) method, in which as little as 500 ng/ml can be detected (12).

In Japan we have been using a reversed passive latex agglutination (RPLA) method for detection of staphylococcal enterotoxins, which has proved to be more sensitive than the gel diffusion methods. In this communication, we report the use of the RPLA method for detection of TSST-1 production by staphylococcal strains isolated from TSS patients and from other sources.

MATERIALS AND METHODS

Bacterial strains. Twenty *Staphylococcus aureus* strains associated with TSS and 21 *S. aureus* strains from non-TSS controls were obtained from James Todd, Department of Pediatrics, Children's Hospital, Denver, Colo. One hundred *S. aureus* strains were obtained from each of the following sources: (i) clinical isolates from various staphylococcal infections supplied by H. Zen-Yogi, Department of Microbiology and Clinical Pathology, School of Medicine, Kyorin University, Tokyo, Japan; (ii) isolates from feces of healthy adults in Tokyo collected in 1982; (iii) strains isolated in connection with staphylococcal food poisoning outbreaks in Tokyo from 1978 to 1982; and (iv) isolates from foods purchased at retail markets in Tokyo from 1981 to 1982.

TSST-1 and enterotoxin reagents. Purified TSST-1 (7) and purified enterotoxins were prepared in the Tokyo Metropolitan Research Laboratory of Public Health. The purity of the toxins was determined to be better than 95% when the purified toxins gave single bands in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Stock solutions of 100 μg of purified toxin per ml (concentrations were determined by A_{280}) were prepared for use in the assays. The specific antisera were prepared by injecting the toxins into rabbits subcutaneously (7) and were purified by the affinity chromatographic method of Yamada et al. (16).

Sensitizing of latex particles. Latex particles (SLD-59; 0.9 μm in diameter) were obtained from Takeda Chemical Industries, Ltd., Osaka, Japan. A 5% suspension of latex particles was diluted 1:10 with phosphate-buffered saline consisting of 1 volume of 67 mM phosphate buffer (pH 7.2) and 3 volumes of saline with 0.05% NaN_3. Equal volumes of a diluted latex particle suspension and 60 μg of anti-TSST-1 immunoglobulin per ml (concentration was determined by A_{280}) were mixed and incubated at 37°C for 30 min. Sensitized latex particles were washed once with phosphate-buffered saline and once with a diluent consisting of 0.83% bovine serum albumin and 0.004% polyvinylpyrrolidine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) in phosphate-buffered saline. The particles were suspended in the diluent at a concentration of 0.025%. Latex particles to be used as controls were sensitized with normal rabbit globulin in the same manner as anti-TSST-1-treated particles. Normal rabbit globulin was purified from normal rabbit sera with protein A-Sepharose CL-4B (Pharmacia, Inc., Piscataway, N.J.). Latex particles were sensitized with the specific antiserum to each of the staphylococcal enterotoxins (SE) (enterotoxins SEA, SEB, SEC, SED, SEE) in the same manner as with anti-TSST-1. The sensitized latex particles were stored at 4°C.

Preparation of supernatant fluids. *S. aureus* strains were grown in 10 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) in a test tube at 37°C for 18 h with a Recipro shaker (Trio Science Co., Ltd., Tokyo, Japan) at 100 rpm. The cultures were centrifuged at 25,000 × *g* at 4°C for 15 min.

RPLA for TSST-1 and enterotoxin detection. Serial 10-fold dilutions of each *S. aureus* supernatant fluid (25 μl per well) were placed in each of two rows of U-type microdilution plate (N-1182; Nunc, Roskilde, Denmark). To each well in one row (e.g., see Fig. 1, row 1a) was added 25 μl of latex particles sensitized with anti-TSST-1 or anti-enterotoxin preparation, and to each well in the second row (e.g., see Fig. 1, row 1b) was added 25 μl of normal rabbit globulin-sensitized latex particles. After thorough mixing, the plates

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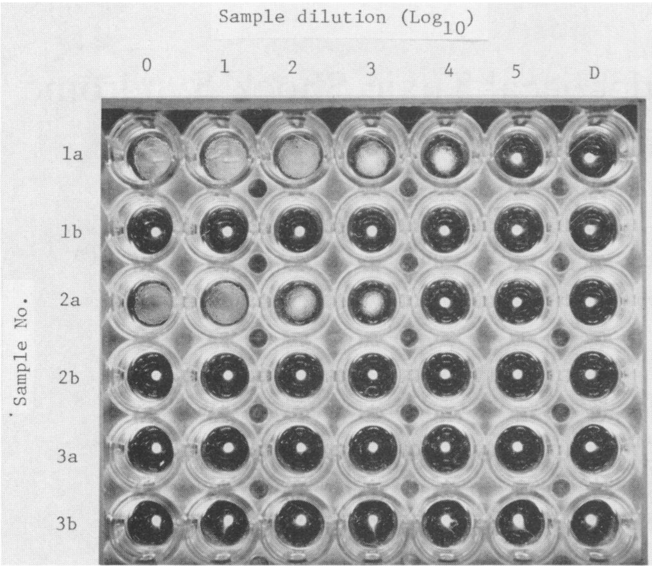

FIG. 1. Analysis for TSST-1 by RPLA. Tenfold serial dilutions of samples were used. Anti-TSST-1-sensitized latex particles are in rows 1a, 2a, and 3a. Normal rabbit globulin-sensitized latex particles are in rows 1b, 2b, and 3b. Purified TSST-1 is in rows 1a and 1b with 10 μg/ml in wells 0, 1 μg/ml in wells 1, 2, etc. Supernatant fluid from a TSST-1 *S. aureus* strain is in rows 2a and 2b with undiluted fluid in wells 0, fluid diluted 10-fold in wells 1, 2, etc. Supernatant fluid from a non-TSST-1-producing *S. aureus* strain is in rows 3a and 3b with undiluted fluid in wells 0, fluid diluted 10-fold in wells 1, 2, etc. The endpoint of the purified TSST-1 in row 1a is well 4, which represents 1 ng of TSST-1 per ml. The endpoint of the positive *S. aureus* strain in row 2a is well 3, which represents 1 μl of supernatant fluid and is equal to 1 ng/μl or 1 μg of TSST-1 per ml of undiluted supernatant fluid.

were incubated in a humidified box for 16 h at room temperature. Agglutination was observed macroscopically with transmitted light through the bottoms of the plates. Positive reactions appeared as irregular formations of agglutinated particles (e.g., see Fig. 1, wells 0 to 4, row 1a) compared with a smooth-contoured sedimentation of particles of a negative reaction (e.g., see Fig. 1, well 5, row 1a). The endpoint of the titration was taken as the last well in the series that showed a detectable difference from a negative control well (e.g., see Fig. 1, well 4, row 1a, and well 3, row 2a). The titers of the samples were determined as the reciprocals of the endpoint dilutions; for example, the titer of the sample in row 2a (Fig. 1) would be 1,000, and in terms of the amount of TSST-1 it would be 1,000 times 1 ng (the detection limit of purified TSST-1 [Fig. 1, well 4, row 1a]) or 1 μg/ml of culture supernatant fluid of the strain used in the test. The minimum concentration of TSST-1 detectable by RPLA was calculated from the titer of purified TSST-1 to be 1 ng/ml (1:10,000 dilution in well 4, row 1a). Similar procedures were followed for the analysis of enterotoxins.

OSP for TSST-1 and enterotoxin testing. TSST-1 and enterotoxin production was also detected by the OSP method (12). The membrane-over-agar method (12) was used for growth of the *S. aureus* strains. In brief, an inoculum of 0.1 ml was placed on a dialysis membrane that had been placed over brain heart infusion agar in a 100-mm petri plate and incubated overnight at 37°C. The culture was removed from the membrane with 2.5 ml of 0.01 M Na_2HPO_4 and centrifuged to remove the cells. The culture supernatant fluid (40 μl per well) was analyzed by the OSP method, which is a modification of the Ouchterlony gel diffusion plate method. The sensitivity of the method is approximately 500 ng/ml.

Concentration of supernatant fluids. Portions (100 ml) of the supernatant fluids collected from strains that were TSST-1 positive by RPLA but negative by the OSP method (incubated in brain heart infusion broth by the test tube method described above) were put in dialysis tubes (Union Carbide Corp., Chicago, Ill.), and the tubes were placed in 50% polyethylene glycol 2000 (Nihon Chromato Co., Ltd., Tokyo, Japan) at 4°C for 18 h. The concentrated fluids were diluted to 1 ml and tested for TSST-1 presence by the OSP method. Also, the supernatant fluids from five strains that were SEA positive by RPLA but negative by the OSP method were concentrated 100-fold, and the concentrated fluids were analyzed for the presence of SEA.

Protein A interference. Protein A (up to 1 mg/ml) (Pharmacia) was dissolved in the supernatant fluid of an *S. aureus* strain that did not produce TSST-1 or in phosphate-buffered saline and tested by RPLA against anti-TSST-1-sensitized latex particles. Also, the culture supernatant fluid of *S. aureus* Cowan I, a high protein A producer, was tested against anti-TSST-1-sensitized latex particles.

RESULTS

RPLA and OSP testing for TSST-1 and enterotoxin production. The results of testing the supernatant fluids from one *S. aureus* strain for the production of TSST-1 versus purified TSST-1 by RPLA are illustrated in Fig. 1, rows 2a and 1a, respectively. For comparison, the culture supernatant fluid from an *S. aureus* strain that did not produce TSST-1 is shown in Fig. 1, row 3a. There was no reaction of the culture supernatant fluids of the *S. aureus* strains with latex particles sensitized with normal rabbit globulin (Fig. 1, rows 2b and 3b). This indicated the reaction with anti-TSST-1-sensitized latex particles to be specific. Actually, none of the strains tested showed any reaction with latex particles sensitized with normal rabbit globulin. None of the enterotoxins (SEA to SEE) showed any cross-reaction with latex particles sensitized with anti-TSST-1. The amount of TSST-1 produced by the *S. aureus* strain (Fig. 1, row 2a) was estimated to be about 1 μg/ml of culture supernatant fluid.

Results of the testing of the 20 TSS strains and the 21 control strains for TSST-1 and enterotoxins are given in Table 1. The supernatant fluids from the three strains that

TABLE 1. Production of TSST-1 and enterotoxins by *S. aureus* strains

Toxin(s)	No. of TSS strains		No. of control strains	
	RPLA	OSP	RPLA	OSP
TSST-1 (total)	17	16	6	4
TSST-1 (alone)	0	4	1	3
TSST-1 + SEA	11	9	1	0
TSST-1 + SEB	0	0	1	0
TSST-1 + SEC	3	3	2	1
TSST-1 + SEA + SED	2	0	1	0
TSST-1 + SEA + SEB + SED	1	0	0	0
SEA	1	0	0	1
SEB	0	0	1	3
SEA + SEB	0	0	3	1
SEA + SED	1	0	4	2
SEB + SEC + SED	0	0	1	0
None	1	4	6	10

were negative for TSST-1 by the OSP method were positive after the fluids were concentrated 100-fold, thus confirming the positive reactions obtained with RPLA. Also, the supernatant fluids from five strains that were negative for SEA production by the OSP method were positive after the fluids were concentrated 100-fold.

Of the 100 *S. aureus* strains from staphylococcal infections, 18 produced TSST-1, whereas only 3 of the 100 strains isolated from the stools of healthy adults in Japan produced TSST-1. Of the 100 strains isolated from foods purchased at retail markets, 7 produced TSST-1, and only 3 of the 100 strains isolated in connection with staphylococcal food poisoning outbreaks produced TSST-1. Two of the latter strains produced SEA and SEC, and the other one produced SEA, the enterotoxin most frequently implicated in this type of food poisoning.

Protein A interference. Protein A dissolved in PBS or the supernatant fluid (up to 1 mg/ml) of an *S. aureus* strain that did not produce TSST-1 did not react with anti-TSST-1-sensitized latex particles. Nor did the supernatant fluid from *S. aureus* Cowan I react with anti-TSST-1-sensitized latex particles.

DISCUSSION

We have shown by the results of this investigation that the RPLA method is a simple and sensitive assay for TSST-1. The test can be read visually, thus eliminating the need for any special equipment for recording the results. Only a small amount of specific antibody is needed to sensitize the latex particles. To further simplify the assays, the sensitized latex particles can be made available commercially. Although the reaction is allowed to proceed overnight, it is possible to get preliminary results in a shorter period of time.

Methods, such as radioimmunoassay (5) and enzyme-linked immunosorbent assay (9), that are equally as sensitive as the RPLA method, have been developed for the assay of TSST-1. However, the disadvantages of the radioimmunoassay are the need for handling radioactive materials and the requirement for sophisticated equipment such as a scintillation counter. In the sandwich enzyme-linked immunosorbent assay, extra amounts of specific antibody are required, and specialized equipment, such as a plate reader, is needed. Admittedly, both the radioimmunoassay and the enzyme-linked immunosorbent assay are quantitative methods, whereas the RPLA method is only semiquantitative. This is not of particular concern, however, because when analyzing strains for the production of TSST-1 it is not required that the analysis be quantitative.

Many *S. aureus* strains produced protein A, which reacts nonspecifically with the Fc portion of antibodies; this has been of some concern in the analysis of enterotoxins by enzyme-linked immunosorbent assay (8). Although the interference can be eliminated by treatment with normal rabbit globulin, we have found that as much as 1 mg of protein A does not interfere in the RPLA method. In any case, the use of latex particles sensitized with normal rabbit globulin helps to indicate any false-positive reactions.

It is not known whether any of the 18 individuals with staphylococcal infections from which TSST-1-producing staphylococci were isolated had any of the signs and symptoms of TSS. One would expect at least some of them to have developed such symptoms, because many TSS cases in the United States have resulted from staphylococcal infections other than those related to the use of tampons during menstruation (9). Analysis of the staphylococci isolated from these patients showed that a high percentage of them produce TSST-1 (2). Certainly many staphylococcal infections would not result in TSS, but if the staphylococci involved produced TSST-1 and the individuals had essentially no antibodies to TSST-1, development of the signs and symptoms of TSS might be expected.

It is intriguing that many more *S. aureus* strains isolated from staphylococcal infections produced TSST-1 (18%) than did the staphylococci isolated from stools of healthy individuals (3%). The low percentage of TSST-1-positive strains obtained from stools, together with the small number of isolates from the other sources that were positive for TSST-1 (5%), indicate that TSST-1-producing staphylococci are not very common in Japan.

Originally, TSST-1 was thought to be an enterotoxin because of the emetic reactions that were observed in monkeys (2), but this reaction could not be confirmed with the purified toxin (11). The toxin was found to be easily digestible by pepsin; hence, it would not survive the conditions existing in the stomach which are essential for the development of staphylococcal food poisoning. In the three cases in which TSST-1-producing staphylococci were isolated from the implicated food, the staphylococci also produced one of the enterotoxins, the known causative agents of staphylococcal food poisoning.

The significance of the production of as little as 1 ng of TSST-1 per ml by *S. aureus* strains is not known; however, the detection of TSST-1 production by RPLA of at least one TSS strain that was negative for TSST-1 by the OSP method may be an important indicator. It could be that some of the strains isolated from TSS patients and reported to be negative for TSST-1 production by the OSP method (2) may be positive by RPLA. It should be noted also that a higher percentage of the TSS strains produced one or more of the enterotoxins as determined by RPLA compared with the OSP method (19 versus 12); only one TSS strain was negative for production of either TSST-1 or enterotoxin by RPLA versus four by the OSP method. It is not known whether enterotoxins contribute to TSS, but they have been shown to produce many of the signs and symptoms of TSS when given intravenously to monkeys (1). Evidence to support this contention has been accumulated in the Food Research Institute, and a paper on this is in preparation.

One fact that should be considered is that TSST-1 strains can be stimulated to produce increased amounts of TSST-1 under various conditions (unpublished data). It is not known how the conditions present in staphylococcal infections, particularly during menstruation with a tampon in place, affect the production of TSST-1, but these conditions may be highly stimulatory for production of the toxin. If this is the case, then those strains that produce very low amounts of TSST-1 in vitro may produce sufficient amounts of the toxin in vivo to produce TSS. To be able to detect these low producers would be advantageous.

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